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INSECT TRANSMISSION, HOST RANGE, AND PROPERTIES OF THE CRINKLE-LEAF STRAIN OF WESTERN-CELERY-MOSAIC VIRUS[1]

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IN THE last five years several viroses affecting celery have been observed to occur naturally in California. These, except western celery mosaic, have been only briefly described in previous papers (Freitag and Severin, 1939; Severin and Freitag, 1938).[4] A mosaic disease apparently different from western celery mosaic was first observed near Milpitas in the Santa Clara Valley during November, 1937. The symptoms resemble those of western celery mosaic except that the leaves are, as a rule, severely crinkled. The disease is not common and has been found only rarely during routine observation of celery fields.

An investigation was undertaken to determine the symptoms, properties, and host range; likewise, the relative ability of various aphid species that breed on celery to transmit the virus. Aphids were compared with mechanical inoculation as a means of transmitting the virus. The retention of the virus by three species of aphids was studied experimentally.

MATERIALS AND METHODS

The virus was obtained from a naturally infected celery plant collected at Milpitas. A continuous supply was maintained in the greenhouse through repeated mechanical inoculation of healthy celery plants by the carborundum method described by Rawlins and Tompkins (1936).

The general methods employed in these studies resemble those described earlier (Severin and Freitag, 1938). The aphid species used were maintained, with two exceptions, on celery in the greenhouse. The green peach aphid, *Myzus persicae* (Sulzer), was reared on sugar beets; and the potato aphid, *Macrosiphum solanifolii* (Ashm.), on squash. The experiments performed were carried out under greenhouse conditions at Berkeley.

SYMPTOMS

The first symptom of the mosaic virus to develop is a clearing of the veins and veinlets on the youngest leaves of infected celery plants about 10 days

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after inoculation. This is followed by a conspicuous yellow vein banding, with green interveinal areas (plate 1, *A*). The yellow bands are at first narrow (plate 1, *A*), but gradually become broader (plate 1, *B*) and diffuse in outline (plate 1, *C*). The vein clearing and vein banding are often limited to the basal portion of leaflets (plate 1, *C*), though the entire leaflet may be affected (plate 1, *B*). The interveinal areas become chlorotic; they gradually coalesce with the yellow vein banding to form enlarged amber-yellow areas (plate 1, *D*) somewhat resembling celery-calico symptoms (Severin and Freitag, 1938).

The most conspicuous symptoms of the disease are the crinkling of the leaflets and the raised green islands or blisterlike elevations (plate 1, *E, F*) within chlorotic areas. The elevations, irregular in shape and size, are often located in the tissue between yellowed veins. The youngest leaves of infected plants develop a downward or upward curl of the leaf margin (plate 1, *F*); the result is a pronounced crinkling.

Young plants experimentally infected with the virus in the greenhouse are stunted. In the later stages of the disease they become chlorotic and frequently die as a result of infection.

HOST RANGE

Experimental Infection.—Young seedlings of the various plants tested were mechanically inoculated with the virus in the greenhouse. Besides celery (*Apium graveolens* L. var. *dulce* DC.) the following plants, all members of the family Umbelliferae, proved susceptible to the virus:

Large Smooth Prague celeriac, *Apium graveolens* L. var. *rapaceum* DC.
Dill, *Anethum graveolens* L.
Salad chervil, *Anthriscus Cerefolium* Hoffm.
Caraway, *Carum Carvi* L.
Coriander, *Coriandrum sativum* L.
Carrot, *Daucus Carota* L. var. *sativa* DC.
White varieties: Short White, White Belgian
Yellow variety: Yellow Belgian
Orange varieties: Chantenay, Chantenay Red Cored, Danvers, Early Scarlet Horn, Imperator, and Long Orange
Long Smooth parsnip, *Pastinaca sativa* L.
Single, or Plain, parsley, *Petroselinum crispum* Nym. var. *latifolium*
Anise, *Pimpinella Anisum* L.

Recovery of Virus.—From all the experimentally infected plants listed above the virus was recovered by extracting juice and inoculating it into healthy celery plants.

Symptoms.—Vein clearing and mottling usually developed as a result of infection. On some plants, chlorotic streaks along the veins and crinkling of the leaves also occurred.

Infection of salad chervil was indicated by stunted chlorotic leaflets with downward-cupped youngest leaves. Brown, sunken necrotic streaks and areas developed along the petioles, followed by marked necrotic lesions on infected leaves.

Carrots were, as a rule, only mildly affected; the symptoms were often indistinct. A few plants, however, showed definite symptoms. First, after

inoculation, the veins cleared; then chlorotic spotting and streaking appeared along them. The irregular chlorotic areas on the leaflets and the sunken dark brown areas on the petioles became necrotic. Infected carrots were often dwarfed and had curled, malformed, dwarfed leaves.

Plants That Failed to Develop Infection.—The following plants, when inoculated with juice from infected celery, failed to develop infection under greenhouse conditions. An attempt was made to recover the virus from all inoculated plants even though they failed to develop symptoms.

Chenopodiaceae
- Sugar beet, *Beta vulgaris* L.
- Long Standing Bloomsdale spinach, *Spinacia oleracea* L. var. *inermis* Peterm.

Compositae
- Lemon King calendula or pot-marigold, *Calendula officinalis* L.
- Whitloof chicory, *Cichorium Intybus* L.
- Orange African daisy, *Dimorphotheca aurantiaca* DC.
- New York lettuce, *Lactuca sativa* L. var. *capitata* Hort.
- Giant White and Scarlet Gem zinnia, *Zinnia elegans* Jacq.

Cruciferae
- February cauliflower, *Brassica oleracea* L. var. *botrytis* L.

Cucurbitaceae
- Early White Spine cucumber, *Cucumis sativus* L.
- Zucchini or Italian Marrow squash, *Cucurbita pepo* L.

Solanaceae
- California Wonder pepper, *Capsicum frutescens* L. var. *grossum* Bailey
- Jimson weed, *Datura Stramonium* L.
- Marglobe tomato, *Lycopersicon esculentum* Mill. var. *commune* Bailey
- Connecticut Seed Leaf, Havana, White Burley, and Turkish tobacco, *Nicotiana Tabacum* L.
- *Nicotiana glutinosa* L.
- Black Beauty and New York Improved Spineless eggplant, *Solanum Melongena* L. var. *esculentum* Nees
- Sutton Flourball potato, *Solanum tuberosum* L.

Umbelliferae
- Poison hemlock, *Conium maculatum* L.
- Cow parsnip, *Heracleum lanatum* Michx.

PROPERTY STUDIES OF VIRUS

Experiments were undertaken to determine certain properties of the virus—thermal inactivation, tolerance to dilution, longevity *in vitro*, and resistance to alcohol. The leaves of experimentally infected celery plants grown in the greenhouse were ground to a plup, and then the juice was expressed through cheesecloth. Five celery plants were inoculated with the juice of each preparation.

Thermal Inactivation.—Experiments to determine the thermal-inactivation temperature were carried out by pipetting 10 cc of leaf juice from experimentally infected celery plants into thin-walled glass test tubes, which were then plugged with cotton. When the water bath had reached the desired temperature, the test tubes were immersed for 11 minutes; 1 minute was allowed for the juice to reach the temperature of the bath. The water was maintained within 1 degree of the desired temperature by turning the electric heating

units on and off as necessary. To maintain a uniform temperature throughout the bath, the water was agitated and kept circulating by a stirring rod attached to an electric motor. After the 11 minutes, the test tubes were cooled in running tap water. The virus was tested at five different temperatures at 5-degree intervals, beginning at 45° C.

TABLE 1

THERMAL INACTIVATION OF CELERY CRINKLE-LEAF-MOSAIC VIRUS

Temperature, ° C	Number of preparations infectious of 7 tested	Celery plants infected of 35 inoculated	
		Number	Per cent
22	7	32	91.4
45	7	35	71.4
50	4	5	14.3
55	1	1	2.9
60	0	0	0.0
65	0	0	0.0

Seven preparations were tested. According to the data in table 1, only one of these was still infectious when heated for 10 minutes at 55° C, and no infection resulted from juice treated at 60° and 65°. These results show that the virus is thermally inactivated between 55° and 60°, though the greatest inactivation occurred between 45° and 50°.

Tolerance to Dilution.—Eight separate preparations were made to test the tolerance of the virus to dilution. The results indicate a low concentration of

TABLE 2

TOLERANCE OF CELERY CRINKLE-LEAF-MOSAIC VIRUS TO DILUTION

Dilution	Number of preparations infectious of 8 tested	Celery plants infected of 40 inoculated	
		Number	Per cent
Control	8	32	80.0
1 : 10	8	31	77.5
1 : 100	5	8	20.0
1 : 1,000	0	0	0.0
1 : 10,000	0	0	0.0
1 : 100,000	0	0	0.0

virus in the experimentally infected plants. As table 2 shows, the 1:10 dilutions were almost as effective as undiluted juice; but the 1:100 dilutions were only about one fourth as effective as the undiluted virus, and infections were obtained with only five of the eight preparations. No infections were obtained with the 1:1,000 or greater dilutions.

Tolerance to Aging in Vitro.—Experiments were conducted to determine the tolerance of the virus to aging *in vitro*. Ten cubic centimeters of juice extracted from the leaves of experimentally infected celery plants were placed in each test tube, which was plugged with cotton and stored at room temperature, about 22° C. Seven separate preparations were inoculated daily for 7

days after the extraction of the juice. Five celery plants were inoculated with each preparation each day.

The results (table 3) show that three preparations were still infectious on the third day, but that all seven were inactivated by the fourth day. The greatest inactivation occurred during the first 24 hours.

TABLE 3

TOLERANCE OF CELERY CRINKLE-LEAF-MOSAIC VIRUS TO AGING *in Vitro*

Days exposed	Number of preparations infectious of 7 tested	Celery plants infected of 35 inoculated	
		Number	Per cent
0 (control)	7	33	94.3
1	2	6	17.1
2	2	3	8.6
3	3	5	14.3
4	0	0	0.0
5	0	0	0.0
6	0	0	0.0
7	0	0	0.0

Resistance to Alcohol Treatment.—Tests were made to determine the resistance of the virus to alcohol during 1-hour exposures. To juice extracted from experimentally infected celery plants, absolute alcohol was added in such quantities that resulting mixtures of 10, 20, 30, 40, and 50 per cent alcohol were obtained. These were allowed to stand for 1 hour. The precipitate that formed was separated from the rest of the mixture by centrifuging for 15 minutes at 3,500 revolutions per minute. The supernatant alcoholic solution was poured from the centrifuging tubes and inoculated into five healthy celery plants in each of five separate preparations. The precipitate was first washed in sterile distilled water, then resuspended in a quantity of distilled water equal to the original volume of the extracted celery juice, and

TABLE 4

RESISTANCE OF CELERY CRINKLE-LEAF-MOSAIC VIRUS EXTRACT TO ALCOHOL DURING ONE-HOUR EXPOSURES

Per cent alcohol	Number of preparations infectious of 5 tested		Celery plants infected of 25 inoculated			
			Number		Per cent	
	Supernatant liquid	Precipitate*	Supernatant liquid	Precipitate	Supernatant liquid	Precipitate
0 (control)	5	5	21	24	84	96
10	1	5	2	22	8	88
20	1	5	1	15	4	60
30	0	2	0	2	0	8
40	0	0	0	0	0	0
50	0	0	0	0	0	0

* The control juice became turbid on standing; and on centrifugation a precipitate was separated, which was resuspended in distilled water.

finally used for inoculation. The control juice became turbid on standing; and on centrifugation a precipitate was separated, which was resuspended in distilled water.

As shown in table 4, the virus in the supernatant liquid was still active in 20 per cent alcohol in one of five preparations, but was inactivated in all preparations tested at 30 per cent alcohol. The virus in the precipitate was active in two of five preparations of 30 per cent alcohol, whereas all preparations tested at 40 per cent alcohol proved inactive. The virus in the supernatant liquid and that in the precipitate probably have the same resistance to alcohol. The fact that there is a higher concentration of the virus in the precipitate might account for the results.

TABLE 5

TRANSMISSION OF CELERY CRINKLE-LEAF-MOSAIC VIRUS BY MECHANICAL INOCULATION, COMPARED WITH TRANSMISSION BY APHIDS*

Aphid species	Aphid transmission			Mechanical inoculation		
	Plants inoculated	Plants infected		Plants inoculated	Plants infected	
		Number	Per cent		Number	Per cent
Celery leaf aphid, *Aphis apigraveolens* Essig....	25	1	4.0	25	21	84.0
Celery aphid, *Aphis apii* Theo.....	25	0	0.0	25	17	68.0
Rusty-banded aphid, *Aphis ferruginea-striata* Essig.....	50	13	26.0	50	47	94.0
Cotton or melon aphid, *Aphis gossypii* Glover..	25	0	0.0	25	19	76.0
Erigeron root aphid, *Aphis middletonii* Thos....	25	0	0.0	25	21	84.0
Yellow willow aphid, *Cavariella capreae* Fab.....	25	0	0.0	25	19	72.0
Potato aphid, *Macrosiphum solanifolii* (Ashm.).	10	0	0.0	10	10	100.0
Lily aphid, *Myzus circumflexus* (Buck.).....	25	3	12.0	25	23	92.0
Foxglove aphid, *Myzus convolvuli* (Kalt.).....	25	1	4.0	25	20	80.0
Green peach aphid, *Myzus persicae* (Sulzer).....	25	5	20.0	25	22	88.0
Honeysuckle aphid, *Rhopalosiphum conii* (Dvd.)	35	1	2.9	35	31	88.6
Total or average.....	..	..	..	295	250	84.7

* The same experimentally infected plants were used as a source of virus for the aphid transmission and for the mechanical inoculation tests. Five healthy plants were inoculated from each infected plant by each method. Virus was recovered by mechanical inoculation from each previously infected plant.

APHID TRANSMISSION OF VIRUS

Comparison of Mechanical Inoculation with Aphid Transmission.—The transmission of the virus from experimentally infected to healthy celery by mechanical inoculation was compared with transmissions by various species of aphids. Ten species found to breed on celery under natural conditions in California, and the potato aphid, were tested for their ability to transmit the virus; these are listed in table 5. Five lots of twenty-five aphids each were transferred from each infected plant tested to five healthy celery plants, and the juice from the infected plant was then inoculated into another lot of five plants.

The results (table 5) indicate that aphids were less efficient than mechanical inoculations in transmitting the virus. Whereas five of the eleven species of aphids tested failed to transmit it, mechanical inoculations made with the juice from the infected plants on which the aphids had fed produced a high per-

centage of infection. The rusty-banded aphid proved to be the most efficient vector, infecting 13 of 50 celery plants, or 26 per cent. Mechanical inoculations resulted in 250 infections of 295 celery plants inoculated, or 84.7 per cent.

Retention of Virus by Aphids.—The three species of aphids demonstrated to be the most efficient vectors of the virus were tested to determine how long they retained the virus. They were bred on infected celery plants. Twenty-five from each infected plant were then transferred to each of five healthy celery plants and to successive healthy plants daily for 3 days. The aphids were confined on the third lot of celery for 1 week. Since a very low percentage of transmission of the virus was obtained, it was considered desirable to

TABLE 6

RETENTION OF CELERY CRINKLE-LEAF-MOSAIC VIRUS BY THREE SPECIES OF APHIDS TRANSFERRED DAILY TO THREE SUCCESSIVE HEALTHY CELERY PLANTS

Aphid	Aphid transmission					Control tests by mechanical inoculation		
	Plants inoculated daily	Plants infected 1st day	Per cent infected 1st day	Plants infected 2d day	Plants infected 3d to 7th day	Plants inoculated	Plants infected	Per cent infected
Rusty-banded aphid, *Aphis ferruginea-striata* Essig	45	4	8.9	0	0	45	39	86.7
Lily aphid, *Myzus circumflexus* (Buck.)	25	3	12.0	0	0	25	24	96.0
Green peach aphid, *Myzus persicae* (Sulzer)	25	2	8.0	0	0	25	19	76.0
Total or average	..	..	..	..	..	95	82	86.3

demonstrate, by mechanical inoculation of juice into healthy plants, the presence of active virus in all celery used as a source of virus.

The results (table 6) demonstrate that of the three aphid species tested all failed to retain the virus for more than 1 day. The rusty-banded aphid infected 8.9 per cent of the celery plants, the green peach aphid 8.0 per cent, and the lily aphid 12.0 per cent, during the first day; but none of the three aphid species infected any plants thereafter. The infected plants on which the aphids were bred proved to be excellent sources of virus. Their juice, when mechanically inoculated into 95 celery plants, resulted in 82 infections, or 86.3 per cent.

DISCUSSION

Celery crinkle-leaf-mosaic virus is considered to be a strain of western-celery-mosaic virus because of similarities of symptoms, host range, properties, and insect transmission. The results obtained with celery crinkle-leaf mosaic are compared with those previously described for western celery mosaic (Severin and Freitag, 1938). It differs in that it develops a yellow mottle with raised blister-like areas and a pronounced crinkling of the leaves, which readily distinguish it from western celery mosaic. The crinkle-leaf strain develops a more pronounced vein clearing and yellow vein banding.

These are often confined to the basal portion of the leaflet, whereas the vein clearing of western celery mosaic usually extends over the entire leaf.

The host ranges of the two strains are apparently limited to plants of the family Umbelliferae. Crinkle-leaf strain was transmitted to Long Smooth parsnip and anise, two host plants to which the western-celery-mosaic virus was not transmitted. Additional inoculations with the latter virus will be necessary, however, before these two hosts can be considered immune to it. Carrot proved more susceptible to western celery mosaic.

The properties of the two viruses may be summarized as follows:

	Celery crinkle-leaf mosaic	Western celery mosaic[5]
Thermal inactivation (10-minute exposure)	60° C	60° C
Tolerance to dilution	1:100	1:4,000
Tolerance to aging *in vitro*	3 days	6 days
Resistance to alcohol		
Supernatant liquid	20 per cent	30 per cent
Precipitate	30 per cent	40 per cent

Transmission of the crinkle-leaf strain by the extracted juice from experimentally infected plants has given a uniformly high percentage of infection. As a rule, the western-celery-mosaic virus was just as readily transmitted; but in certain instances the percentage of plants infected dropped to less than 50 per cent.

Aphids proved inefficient as vectors of the crinkle-leaf strain. Five of eleven species tested failed to transmit the virus, while the six species that were successful infected only 4 to 26 per cent of the celery plants on which they fed. Western-celery-mosaic virus was readily transmitted by all the aphid species tested. The six species known to breed on celery and proved to be capable of transmitting both viruses were more efficient for the western celery mosaic, infecting 53 to 92 per cent of the plants on which they fed.

[5] Data from Severin and Freitag, 1938.

SUMMARY

The host range of celery crinkle-leaf-mosaic virus is limited to plants belonging to the family Umbelliferae. The following have been experimentally infected: celery, *Apium graveolens* L. var. *dulce* DC.; Large Smooth Prague celeriac, *Apium graveolens* L. var. *rapaceum* DC.; dill, *Anethum graveolens* L.; salad chervil, *Anthriscus Cerefolium* Hoffm.; caraway, *Carum Carvi* L.; coriander, *Coriandrum sativum* L.; Long Smooth parsnip, *Pastinaca sativa* L.; Single, or Plain, parsley, *Petroselinum crispum* Nym. var. *latifolium;* anise, *Pimpinella Anisum* L.; and nine varieties of carrot, *Daucus Carota* L. var. *sativa* DC.

Symptoms of celery crinkle-leaf-mosaic virus consist of yellow mottling, leaf crinkling, raised blisterlike areas, and a pronounced vein clearing.

The properties of the virus were found to be as follows: The thermal inactivation is 60° C in 10-minute exposures. The tolerance to dilution is 1:100. The virus remains active in extracted celery juice kept in a test tube at room temperature for 3 days. It is not completely inactivated in the supernatant liquid of a 20 per cent alcohol mixture nor in the precipitate of a 30 per cent alcohol mixture during 1-hour exposures.

The virus was more readily transmitted by mechanical inoculation than by aphid vectors. Mechanical inoculations of extracted celery juice produced infection in 84.7 per cent of the celery plants inoculated. Only 6 of 11 species of aphids tested proved capable of transmitting the virus, and these infected only 8.1 per cent of the plants inoculated.

Three species of aphids retained the virus the first 24 hours, but failed to infect a second lot of plants the next day or a third lot the following week.

Results of studies of host ranges, properties, and transmission of viruses by aphids and mechanical inoculation indicate that celery crinkle-leaf mosaic is a strain of western-celery-mosaic virus.

LITERATURE CITED

FREITAG, J. H., and H. H. P. SEVERIN.
1939. Additional celery viroses. (Abstract.) Phytopathology **29**:824.

RAWLINS, T. E., and C. M. TOMPKINS.
1936. Studies on the effect of carborundum as an abrasive in plant virus inoculations. Phytopathology **26**:578–87.

SEVERIN, H. H. P., and J. H. FREITAG.
1938. Western celery mosaic. Hilgardia **11**(9):493–558.

Plate 1.—Symptoms of celery crinkle-leaf mosaic on leaflets of celery from plants experimentally infected by mechanical inoculation: *A*, vein clearing with narrow yellow veinbanding and green interveinal areas; *B*, vein clearing and broad yellow veinbanding with chlorotic interveinal areas; *C*, diffuse yellow veinbanding and enlarged chlorotic interveinal areas limited to the basal portion of the leaflets; *D*, enlarged chlorotic areas formed by the coalescing of yellow veinbanded areas and the interveinal chlorotic areas; *E*, crinkling and green blisterlike elevations within chlorotic areas; *F*, malformation, curling, crinkling, and large chlorotic areas with green blisterlike elevations.

TRANSMISSION OF CELERY-YELLOW-SPOT VIRUS BY THE HONEYSUCKLE APHID, RHOPALOSIPHUM CONII (DVD.)

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TRANSMISSION OF CELERY-YELLOW-SPOT VIRUS BY THE HONEYSUCKLE APHID, RHOPALOSIPHUM CONII (DVD.)[1]

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CELERY YELLOW SPOT was first observed during the summer of 1934 in the Santa Clara Valley near Milpitas and has since been found in the celery fields near San Jose, Hollister, Salinas, and Sacramento. Symptoms have been briefly described (Severin and Freitag, 1938),[4] and results of insect-transmission experiments (Freitag and Severin, 1939) presented, in previous papers. The disease causes no appreciable loss to celery growers, although 40 per cent of the plants were infected in some fields. Because the plants are only slightly stunted and because the spotted outer leaves are normally discarded in the harvesting of celery for the market, the disease is of small economic importance.

In 1935 an investigation was undertaken to study the symptoms and host range of celery-yellow-spot virus. Attempts were made to transmit the virus by means of different aphid species and by mechanical inoculations. Virus transmission by single specimens of winged and mature wingless aphids and retention by the aphid vector were the subject of experiment.

SYMPTOMS

Usually the first symptom noticed in the greenhouse, about 14 days after inoculation of celery plants by the honeysuckle aphid, *Rhopalosiphum conii* (Dvd.) [*R. melliferum* (Hottes)], was the irregular pale green areas or spots and stripes, which rapidly became yellow (plate 1, *B*). This yellow spotting is the most characteristic sign of the disease. The spots and stripes are mostly along the veins (plate 1, *C*; plate 2, *A*), but are also scattered irregularly over the leaflets (plate 1, *E*; plate 2, *E*).

The yellow areas are irregular in shape and variable in size (plate 1, *D*; plate 2, *B, C*). The spots along the veins are often elongate (plate 1, *C*; plate 2, *E*) and sometimes occur at the basal portion of veinlets, where the latter join the main and lateral veins (plate 2, *A*). Some of the yellow spots are round and form small circular chlorotic areas (plate 2, *D*). The chlorotic spots may be numerous (plate 2, *D*) and may result in a general yellowing of the leaflet (plate 1, *E*). In advanced stages of the disease the small chlorotic spots may coalesce, forming enlarged spots (plate 1, *D*; plate 2, *B, D*) and mottled areas (plate 1, *F*). Their yellow color gradually fades and may become white as the leaf matures.

The petioles of naturally infected celery plants develop circular white spotting (fig. 1). When the epidermis is removed from these areas, brown specks may be seen along the veins of the celery stalk (plate 2, *F*).

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Fig. 1.—Petioles from celery plants: left, petiole from a healthy plant; right, three petioles from celery naturally infected with yellow spot, showing white spotting. (Milpitas, October 17, 1935.)

HOST RANGE

Natural Infection.—Celery (*Apium graveolens* L. var. *dulce* DC.), poison hemlock (*Conium maculatum* L.), and parsnip (*Pastinaca sativa* L.) are the only plants that were found to be naturally infected with celery yellow spot. Virus was recovered, by the honeysuckle aphid, from naturally infected parsnip and poison hemlock. Although the virus has not yet been recovered from celery either by aphids or by mechanical inoculation (see p. 380), the symptoms on naturally infected celery are identical with those on celery experimentally infected by the honeysuckle aphid from infected poison hemlock and parsnip.

Naturally infected parsnip plants, found at Stockton, showed a mild mottling of the leaves, whereas poison hemlock proved to be a symptomless carrier.

Experimental Infection of Celery.—Experiments were conducted to infect celery with yellow spot. Flower clusters from poison-hemlock plants heavily infested with honeysuckle aphids were collected from several localities. None of the plants from which the aphids were taken manifested yellow-spot symptoms. Poison hemlock that showed mottling, spotting, chlorotic rings, and line patterns was always demonstrated to be infected with the poison-hemlock-ringspot virus (Freitag and Severin, 1945). Plants, however, rarely suffered from a virus complex of ringspot and yellow spot. The poison-hemlock flower clusters infested with aphids were cut so that each piece had 50 to 100 aphids on it. From 5 to 10 pieces were cut from a single infested plant; then each piece was placed in a cage with a healthy celery plant. As the flower clusters wilted and dried, the aphids would move to the healthy celery leaves. There they fed for 2 to 5 days, after which they were fumigated with Nico-fume tobacco paper. Samples were taken near Alvarado, Sacramento, San Pablo, and San Jose from 25 poison-hemlock plants which proved to be naturally infected. The results obtained were as follows:

Locality and poison-hemlock plant no.	Celery plants inoculated	Celery plants infected
San Pablo		
No. 1	10	10
No. 2	10	6
No. 3	10	1
San Jose		
No. 4	10	2
Sacramento		
No. 5	5	5
No. 6	5	3
No. 7	5	3
No. 8	5	2
No. 9	5	1
Alvarado		
No. 10	10	10
No. 11	10	10
No. 12	10	10
No. 13	10	9
No. 14	10	7
No. 15	10	7
No. 16	10	6
No. 17	10	6
No. 18	10	6
No. 19	10	5
No. 20	10	4
No. 21	10	2
No. 22	5	3
No. 23	5	3
No. 24	5	3
No. 25	5	2
Totals	205	126

These data show that the aphids infected 126 of 205 celery plants, or 61.5 per cent. Transmission by aphids was erratic; the number of plants infected ranged from 10 to 100 per cent. This variation may have resulted from differences in virus concentration in the infected plants; or perhaps the aphids varied greatly in their ability to acquire and transmit the virus.

Experimental Infection of Poison Hemlock and Celery.—Since poison hemlock is a symptomless carrier of the yellow-spot virus, celery plants were inoculated in these experiments to serve as indicators of the presence of the virus. The honeysuckle aphids used for this purpose came from the same source as those used to infect the poison hemlock. They were collected on

TABLE 1

EXPERIMENTAL INFECTION OF CELERY AND POISON HEMLOCK WITH CELERY-YELLOW-SPOT VIRUS BY HONEYSUCKLE APHIDS FROM NATURALLY INFECTED POISON HEMLOCK

Naturally infected poison hemlock no.	Celery plants		Poison-hemlock plants	
	Inoculated	Infected	Inoculated	Infected*
1	10	7	5	3
2	10	5	5	1
3	10	4	5	2
4	10	2	10	0
5	10	1	2	0
6	5	3	5	3
7	5	3	5	0
8	5	3	5	0
9	5	3	5	5
10	5	3	2	0
11	5	2	2	0
12	5	1	3	0
Total	85	37	54	14
Percentage	..	*43.5*	..	*25.9*

* As determined by recovery of the virus by aphids and transfer to healthy celery plants.

naturally infected poison hemlock, and those from each plant were transferred to from 2 to 10 healthy poison hemlock and 5 to 10 healthy celery plants. About a month after the celery had developed symptoms, noninfective honeysuckle aphids were taken to the inoculated poison hemlock, where they were allowed to feed for about a week. Then they were moved to 5 healthy celery plants. Infection of one or more celery plants was considered proof that the poison hemlock was infected, although the latter displayed no symptoms.

As table 1 shows, the aphids were apparently able to infect celery more readily than poison hemlock. In each of 12 trials conducted, they infected 1 to 7 celery plants, whereas other aphids from the same sources infected poison hemlock in only 6 of 12 trials. A total of 37 of 85, or 43.5 per cent, of the celery plants and 14 of 54, or 25.9 per cent, of the poison-hemlock plants became infected.

RECOVERY OF VIRUS FROM POISON HEMLOCK AFTER INOCULATION

Experiments were conducted to test poison hemlock as a source of virus. Inoculations were made in the usual way by transferring honeysuckle aphids

Poison-hemlock plant no. and date of recovery test	Celery plants infected, of 5 inoculated
No. 1 (inoculated June 18); recovery of virus on:	
July 18	2
August 16	0
No. 2 (inoculated June 18); recovery of virus on:	
July 15	1
August 17	3
October 10	2
November 25	0
December 9	0
December 21	0
January 10	0
No. 3 (inoculated June 18); recovery of virus on:	
July 18	1
August 17	0
No. 4 (inoculated June 18); recovery of virus on:	
July 15	5
August 9	0
October 14	0
December 9	0
December 21	0
No. 5 (inoculated June 18); recovery of virus on:	
July 15	3
August 17	0
No. 6 (inoculated June 18); recovery of virus on:	
July 15	2
August 17	0
No. 7 (inoculated June 18); recovery of virus on:	
July 15	1
August 17	0
No. 8 (inoculated September 24); recovery of virus on:	
January 4	2
February 12	1
March 22	0
April 13	2
No. 9 (inoculated August 18); recovery of virus on:	
September 11	0
September 22	1
October 16	1
No. 10 (inoculated August 18); recovery of virus on:	
September 11	0
September 21	5[5]
October 16	0
Total	32
Percentage	19.2

[5] Out of 10 celery plants inoculated.

from naturally infected plants to healthy plants grown from seeds. These plants, as in previous experiments, failed to develop symptoms. The first recovery tests were conducted 24 days after the inoculation. Additional recovery tests were made at irregular intervals. The results were as shown on the preceding page.

The data indicate that experimentally infected poison hemlock is a poor source of the virus. In 6 of 10 tests the aphids recovered the virus from poison hemlock the first month after inoculation, but failed to recover it thereafter. The aphids infected only 32 of 165 celery plants, or 19.4 per cent. The low percentage of transmission obtained by using experimentally infected poison hemlock as a source of virus made it difficult to conduct many experiments. Why the aphids are unable to recover the virus readily from experimentally infected poison hemlock, whereas they were far more successful in acquiring it from naturally infected plants, is not understood.

FAILURE TO RECOVER VIRUS FROM CELERY

Nine species of aphids (listed in table 2) were reared on celery that showed symptoms of yellow spot. The aphids from each infected plant were transferred in lots of 25 to each of 5 healthy celery plants. After this transfer, the juice of the infected plants was extracted by crushing the leaves in a mortar with a pestle and pressing the juice through cheesecloth by hand. Five healthy celery plants were then inoculated by dusting the leaves with powdered carborundum and lightly rubbing the leaves with absorbent cotton that had been dipped in the extracted juice according to the method described by Rawlins and Tompkins (1936).

The transmission results with aphids and with mechanical inoculations are summarized in table 2. As this experiment conclusively demonstrates, the aphids were not able to recover virus from 66 infected celery plants and consequently did not infect any of the 330 celery plants to which they were transferred. Mechanical inoculation of 285 celery plants with the juice from plants used as a source of virus for the aphid tests resulted in no infections.

TRANSMISSION OF VIRUS BY SINGLE APHIDS

A comparison was made of the transmission of yellow-spot virus by single specimens of winged and mature wingless honeysuckle aphids reared on naturally infected poison hemlock. The aphids were collected at Alvarado on poison-hemlock plants of this kind. Each was transferred singly, by means of a camel's-hair brush, to healthy celery and fed for 1 day.

The winged honeysuckle aphids proved to be more efficient vectors than the mature wingless ones. The former infected 20 out of 50 celery plants; the latter only 6 out of 50.

RETENTION OF VIRUS BY APHIDS

Some plant viruses transmitted by aphids are retained, whereas others are lost soon after the aphids leave the infected plants. Experiments were conducted to determine how long the honeysuckle aphids retained the yellow-spot virus. The aphids were collected on naturally infected poison hemlock and transferred with a camel's-hair brush to successive healthy celery plants

daily for 6 to 23 days. Five experiments were performed; and in each, from 10 to 25 lots of 25 aphids were transferred daily to healthy celery, a total of 85 lots being used. Every day, when the aphids were thus transferred, several would die, so that after 10 days only a few remained alive.

TABLE 2

ATTEMPTS TO TRANSMIT CELERY-YELLOW-SPOT VIRUS FROM CELERY TO CELERY BY APHIDS AND BY MECHANICAL INOCULATION

Aphid	Number of infected celery plants tested	Aphid transmission		Mechanical inoculation	
		Plants inoculated	Plants infected	Plants inoculated	Plants infected
Celery aphid, *Aphis apigraveolens* Essig	5	25	0	25	0
Celery aphid, *Aphis apii* Theo.	8	40	0	35	0
Rusty-banded aphid, *Aphis ferruginea-striata* Essig	8	40	0	35	0
Cotton or melon aphid, *Aphis gossypii* Glover	9	45	0	35	0
Erigeron root aphid, *Aphis middletonii* Thos.	5	25	0	25	0
Yellow willow aphid, *Cavariella capreae* (Fab.)	8	40	0	35	0
Lily aphid, *Myzus circumflexus* (Buck.)	8	40	0	35	0
Foxglove aphid, *Myzus convolvuli* (Kalt.)	5	25	0	25	0
Honeysuckle aphid, *Rhopalosiphum conii* (Dvd.)	10	50	0	35	0
Total	66	330	0	285	0

TABLE 3

RETENTION OF CELERY-YELLOW-SPOT VIRUS BY LOTS OF 25 NATURALLY INFECTED HONEYSUCKLE APHIDS TRANSFERRED TO SUCCESSIVE HEALTHY CELERY PLANTS DAILY

Number of days after transfer of aphids from infected plants	Experiment 1		Experiment 2		Experiment 3		Experiment 4		Experiment 5	
	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected
1	25	21	20	17	10	2	20	13	10	7
2	25	9	20	18	10	0	20	13	10	2
3	25	11	20	18	10	1	20	16	10	3
4	25	21	20	18	10	1	20	12	10	8
5	25	20	20	13	10	2	20	10	10	1
6	25	20	20	3	10	0	20	9	10	2
7	..	..	20	0	10	0	20	3	10	0
8	..	..	20	1	10	0	20	2	10	0
9	..	..	..	..	10	0	20	4	10	2
10	..	..	..	..	10	0	20	1	10	3
11	..	..	..	..	..	..	20	1	9	0
12	..	..	..	..	..	..	10	0	7	1
13	..	..	..	..	..	..	3	0	7	0
14	..	..	..	..	..	..	2	0	7*	0

* Continued for 23 days; but no infections resulted after twelfth day.

The results, shown in table 3, indicate that the aphids retain the virus for some time. Apparently, therefore, the mode of transmission differs from that observed for most aphid-transmitted viruses; most aphid vectors fail to transmit after the first day. The honeysuckle aphid retained the virus 5 to 12 days

after being removed from infected poison hemlock. The possibility of aphids' reacquiring virus from plants on which they were allowed to feed for 24 hours was eliminated, since all tests so far conducted with aphids or with mechanical inoculations have failed to recover the virus from infected celery.

DISCUSSION

Judging from the results presented, honeysuckle aphids that have acquired the yellow-spot virus may retain it for 12 days. Soon after being removed from an infected plant, most aphids lose the capacity to produce infection. Several species of them, however, retain viruses for 3 to 29 days. Smith (1929, 1931) and Elze (1927) have shown that the green peach aphid, *Myzus persicae* (Sulzer), the vector of potato-leafroll virus, can feed on immune plants such as cabbage and spinach for 7 to 10 days and then infect healthy potatoes. Bennett (1927) has demonstrated that the raspberry-leaf-curl virus remains active for 2 weeks in the body of the aphid vector, *Aphis rubiphila,* Patch. Osborn (1935, 1938) found that the pea-mosaic virus was retained by the pea aphid, *Macrosiphum onobrachis* (B. d. F.) (*M. pisi* Kalt.), for 29 days and by the potato aphid, *M. solanifolii* (Ashm.) for 21 days. In the experiment of Roland (1939) and Watson (1940) the green peach aphid when transmitting the sugar-beet-yellows virus produced infection on successive healthy plants for a period of 3 days.

Why the aphids were not able to acquire virus from celery plants infected with yellow spot is difficult to explain. A possible reason might be low virus concentration in the celery plant, or plant-tissue relations that make it impossible for aphids to acquire the virus. Hoggan (1929, 1931, 1934) found that aphids did not transmit the tobacco-mosaic virus from one tobacco plant to another, but could acquire it regularly from tomato and transmit it to tobacco. The results obtained with the yellow-spot virus indicate a somewhat similar situation. Recovery of virus from some host plants infected with aster yellows has also proved difficult. Severin and Hassis (1934) were able to infect potato plants with aster-yellows virus by means of the aster leafhopper, *Macrosteles divisus* (Uhl.), but not to recover the virus subsequently from those plants. More recently, however, Severin (1940) reports that aster-yellows virus was recovered from naturally infected potato plants by means of the long-winged aster leafhopper.

SUMMARY

Celery (*Apium graveolens* L. var. *dulce* DC.), poison hemlock (*Conium maculatum* L.), and parsnip (*Pastinaca sativa* L.) were found to be naturally infected with the celery-yellow-spot virus.

The symptoms on celery are irregular areas or spots; or stripes at first pale green, later yellow, and finally white. These spots occur along the veins and scattered over the leaflets.

The virus was recovered from 25 naturally infected poison-hemlock plants, which were symptomless carriers of the disease, and was transmitted by means of the honeysuckle aphid, *Rhopalosiphum conii* (Dvd.), to 126 of 205 celery plants, or 61.5 per cent. Attempts to transmit the virus from infected poison-hemlock plants to healthy plants by mechanical inoculation were unsuccessful.

Attempts to transmit the virus from celery to celery by means of 9 species

of aphids and by mechanical inoculation failed to produce infection in 615 celery plants tested.

Honeysuckle aphids collected on naturally infected poison hemlock transmitted the disease to 37 of 85 celery plants, or 43.5 per cent; and 14 of 54 poison-hemlock plants, or 25.9 per cent.

Experimentally infected poison hemlock proved to be a poor source of virus for aphid-transmission experiments, especially if recovery was attempted after the plant had been diseased for more than a month.

Single specimens of winged honeysuckle aphids proved to be more efficient vectors than mature wingless aphids.

Honeysuckle aphids collected on naturally infected poison-hemlock plants and transferred to successive healthy celery plants daily were able to infect plants for a period of 12 days.

LITERATURE CITED

Bennett, C. W.
1927. Virus diseases of raspberries. Michigan Agr. Exp. Sta. Tech. Bul. **80**:1–38.

Elze, D. L.
1927. De verspreiding van virusziekten van de aardappel (*Solanum tuberosum* L.) door insecten. [Wageningen] Landboushoogesch. (Meded.) **32**:1–90.

Freitag, J. H., and H. H. P. Severin.
1939. Additional celery viroses. (Abstract.) Phytopathology **29**:824.
1945. Poison-hemlock-ringspot virus and its transmission by aphids to celery. Hilgardia **16**(8):389–410.

Hoggan, Isme A.
1929. The peach aphid, *Myzus persicae* (Sulzer), as an agent in virus transmission. Phytopathology **19**:109–23.
1931. Further studies on aphid transmission of plant viruses. Phytopathology **21**:199–212.
1934. Transmissibility by aphids of the tobacco mosaic virus from different hosts. Jour. Agr. Research **49**:1135–42.

Osborn, H. T.
1935. Incubation period of pea mosaic in the aphid *Macrosiphum pisi*. Phytopathology **35**:160–77.
1938. Incubation period of pea virus 1 in the aphid *Macrosiphum solanifolii*. Phytopathology **38**:749–54.

Rawlins, T. E., and C. M. Tompkins.
1936. Studies on the effects of carborundum as an abrasive in plant virus inoculations. Phytopathology **26**:578–87.

Roland, G.
1939. Onderzoekingen verricht in 1937 over de vergelingsziekte en enkele minerale gebreken bij biet en de spinazie. Tijdschr. over Plantenziekten **45**(1):1–22.

Severin, H. H. P.
1940. Potato naturally infected with California aster yellows. Phytopathology **30**:1049–51.

Severin, H. H. P., and J. H. Freitag.
1938. Western celery mosaic. Hilgardia **11**(9):493–558.

Severin, H. H. P., and F. A. Hassis.
1934. Transmission of California aster yellows to potato by *Cicadula divisa*. Hilgardia **8**(10):327–35.

Smith, K. M.
1929. Studies on potato virus diseases. V. Insect transmission of potato leafroll. Ann. Appl. Biol. **16**:209–29.
1931. Studies on potato virus diseases. IX. Some further experiments on insect transmission of potato leafroll. Ann. Appl. Biol. **18**:141–57.

Watson, M. A.
1940. Studies on the transmission of sugar-beet-yellows virus by the aphis, *Myzus persicae* (Sulz.). Roy. Soc. London Proc., Ser. B. **128**:535–52.

Plate 1.—*A*, Healthy celery leaflet. *B* to *F*, symptoms of celery yellow spot on leaflets of celery plants experimentally infected by the honeysuckle aphid, *Rhopalosiphum conii* (Dvd.): *B*, early stage with only a few chlorotic spots between the veins and a few short narrow chlorotic stripes along the veins; *C*, elongated yellow stripes along the veins and chlorotic spotting; *D*, enlarged chlorotic spots, the result of the coalescing of smaller ones; *E*, numerous chlorotic spots with general yellowing of basal portion of leaflets; *F*, coalescing of chlorotic spots to form enlarged yellow areas.

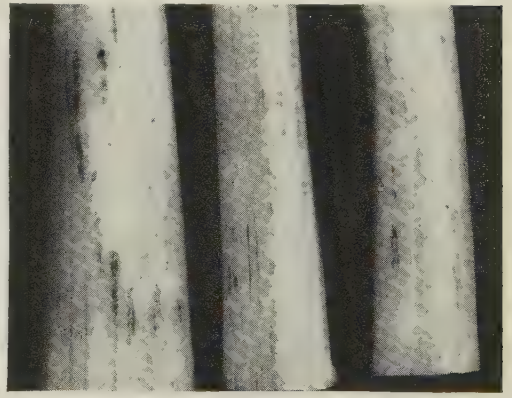
A

B

C

D

E



F

Plate 2.—Symptoms of celery yellow spot on leaflets and petioles of celery taken from naturally infected plants: *A*, yellow spots and stripes along the veins, mostly at basal portion of veinlets where they join main and lateral veins; *B*, irregularly shaped chlorotic spots, many the result of smaller spots coalescing; *C*, small chlorotic spots and stripes along the veins; *D*, numerous small chlorotic spots, some of which are circular; basal portion of leaflet shows enlarged chlorotic spots formed by the coalescing of smaller ones; *E*, chlorotic spotting and elongated stripes along the veins; *F*, epidermis removed from three petioles, showing brown specks along the veins. (Milpitas, November 18, 1934; courtesy M. W. Gardner.)

POISON-HEMLOCK-RINGSPOT VIRUS AND ITS TRANSMISSION BY APHIDS TO CELERY

JULIUS H. FREITAG AND HENRY H. P. SEVERIN

POISON-HEMLOCK-RINGSPOT VIRUS AND ITS TRANSMISSION BY APHIDS TO CELERY[1]

JULIUS H. FREITAG[2] AND HENRY H. P. SEVERIN[3]

INTRODUCTION

DURING an attempt to find a weed reservoir of the western celery mosaic, another virus affecting celery was recovered from one of the weeds tested. Poison hemlock, *Conium maculatum L.*, a common umbelliferous weed, was often found naturally infected with a ringspot virus. The symptoms of ringspot on celery have been described briefly in a previous paper (Severin and Freitag, 1938).[4] Celery plants showing symptoms resembling ringspot were collected in celery fields on several occasions, but attempts to recover virus from these apparently naturally infected plants have failed. The ringspot virus occurs commonly on poison hemlock in the Santa Clara, San Benito, Salinas, and Sacramento valleys of California.

Experiments were undertaken during 1936 to determine the symptoms, host range, and insect vectors of the poison-hemlock-ringspot virus. Various phases of its transmission by aphids were studied, including the relative importance of the different species as vectors, transmission during short feeding periods, retention of the virus by aphids, loss and recovery of infectivity by aphids on celery, and ability of aphids to acquire virus from plants after infection. Experiments were conducted on mechanical transmission of the virus.

A number of virus diseases that produced ringspot symptoms have been described on the following host plants:

Tobacco: Fromme, Wingard, and Priode (1927); Henderson and Wingard (1931); J. Johnson (1936); E. M. Johnson (1930); Price (1936); Priode (1928); Valleau (1932); Wingard (1928)

Potato: Burnett and Jones (1931); J. Johnson (1925); J. H. Smith (1928); K. M. Smith (1929, 1931); Valleau and Johnson (1930)

Tomato: Bald and Samuel (1931); Gardner, Tompkins, and Whipple (1935); Samuel, Bald, and Pittman (1930); K. M. Smith (1932)

Delphinium: Burnett (1934); Valleau (1932)

Clover: Henderson (1934); E. M. Johnson (1933)

Rose: Nelson (1930); White (1930)

Sugar beet: Hoggan (1933)

Hyoscyamus niger L.: Hamilton (1932)

Cabbage: Tompkins, Gardner, and Thomas (1938)

Dahlia: Brierley (1933)

Plum and peach: Valleau (1932)

Peony: Whetzel (1915)

Johnson and Valleau (1935) reviewed the literature on virus diseases causing ringspot symptoms, but without mentioning any virus that causes chlorotic or necrotic ring and line patterns on celery.

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[4] See "Literature Cited" for complete data on citations, referred to in the text by author and date of publication.

Wingard (1928) could not transmit tobacco-ringspot virus to carrot and parsnip, the only two species of umbelliferous plants tested. However, he experimentally infected a total of 62 species of plants belonging to 38 genera in 17 families with the tobacco-ringspot virus.

Wellman (1934) transmitted tobacco-ringspot virus to celery and described the resultant patterns, consisting of small green and yellow spots, many with a small necrotic area. The plants became yellow; and the leaves were malformed, or "shoestring." When southern-celery-mosaic virus was transmitted to Turkish or Broadleaf tobacco, white spots were produced on the inoculated leaves, and, as the plants became older, chlorotic and necrotic zigzag lines and ring patterns developed.

Gardner, Tompkins, and Whipple (1935) demonstrated that celery is susceptible to tomato-spotted-wilt virus, which produces the ringspot symptoms on various host plants. Severin and Freitag (1938) included a description and figure of celery infected with tomato-spotted-wilt virus, but did not record the development of chlorotic rings.

Tompkins, Gardner, and Thomas (1938) noted in cabbage a virus disease that produces necrotic rings. They were unable to infect celery with the cabbage-black-ring virus.

METHODS AND MATERIALS

The source of the ringspot virus used in the experiments here reported was naturally infected poison hemlock collected at Alvarado, California. The virus was transmitted from these plants to healthy ones by the honeysuckle aphid, *Rhopalosiphum conii* (Dvd.) [*R. melliferum* (Hottes)].

The methods used in rearing noninfective aphids and transferring them from one plant to another resemble those reported earlier (Severin and Freitag, 1938).

The mechanical inoculations were performed by dusting leaves with carborundum and then drawing a cotton swab soaked with infective juice over the leaf according to the method of Rawlins and Tompkins (1936).

HOST RANGE

Natural Infection.—Poison hemlock, *Conium maculatum* L., a tall, branching biennial with finely cleft leaflets, is the only plant known to be infected with ringspot in nature. Extremely high populations of the honeysuckle aphid occur on this introduced weed, which is widely distributed throughout California. The umbels or flower clusters are often black with aphids. Honeysuckle aphids, taken from the umbels of naturally infected poison hemlock collected near Alvarado, were used to transmit the virus to healthy celery grown from seed. Lots of 25 to 100 aphids transferred from 15 naturally infected poison-hemlock plants infected 55 of 95 celery plants, or 57.9 per cent.

The virus was recovered from this experimentally infected celery by previously noninfective honeysuckle aphids and transmitted to healthy poison hemlock and celery grown from seed. Lots of 25 aphids were transferred from each of 12 experimentally infected celery plants. They transmitted the virus to 19 of 30 poison-hemlock plants, or 63.3 per cent; and to 29 of 60 celery plants, or 48.3 per cent.

Experimental Infection.—The following economic plants of the family Umbelliferae have been infected by means of the honeysuckle aphid:

Celery, *Apium graveolens* L. var. *dulce* DC.
Large Smooth Prague celeriac, *Apium graveolens* L. var. *rapaceum* DC.
Dill, *Anethum graveolens* L.
Salad chervil, *Anthriscus Cerefolium* Hoffm.
Coriander, *Coriandrum sativum* L.
Carrot, *Daucus Carota* L. var. *sativa* DC.
White varieties: Short White, White Belgian, White Mastodon
Yellow variety: Yellow Belgian
Orange varieties: Chantenay, Chantenay Red Cored, Danvers Half Long, Early Scarlet Horn, French Forcing, Imperator, Improved Long Orange, Nantes, and Oxheart
Hollow Crown and Long Smooth parsnip, *Pastinaca sativa* L.
Single, or Plain, parsley, *Petroselinum crispum* Nym. var. *latifolium*
Double Curled, Extra Triple Curled, and Fern Leaf parsley, *Petroselinum crispum* Nym.
Hamburg, or turnip-rooted, parsley, *Petroselinum hortense* Hoffm. var. *radicosum* Bailey

Recovery of Virus.—The virus, recovered from the experimentally infected plants by previously noninfective honeysuckle aphids, was transferred to celery. Attempts were also made to recover it from inoculated plants that failed to develop symptoms.

SYMPTOMS ON VARIOUS HOSTS

Since poison hemlock is naturally infected with this disease and celery is subject to several virus diseases that might be confused with this one, the symptoms on these two plants are described in detail. Those on other host plants are briefly summarized.

Poison Hemlock.—The first symptoms on the intermediate leaves of experimentally infected plants are usually irregular, scattered, small, pale-green areas, which may enlarge. Later some of the pale-green areas become chlorotic (plate 1, *B*) and may develop minute necrotic centers. Many irregular chlorotic areas of various sizes (plate 1, *C*) appear more commonly along the margin or apical region than near the basal portion of the leaves. Numerous chlorotic patterns are observed on the leaves, such as irregular lines or bands (plate 1, *D*) or zigzag lines resembling somewhat the oak-leaf pattern (plate 1, *E*). Often the chlorotic lines enclose green areas (plate 1, *F*), forming ringspots. The line patterns and chlorotic areas may develop simultaneously. The veins of the leaflets usually remain green, often banded with green tissue (plate 1, *C*), although the remaining leaf tissue may be chlorotic or white. The chlorotic areas often become buff-colored, and sometimes a purplish discoloration of the leaves develops. The two symptoms most useful in identifying this ringspot have been the chlorotic areas and the line patterns.

Under natural conditions the infected plants are not stunted, but often show a downward curling of the leaflets along the midrib. They can easily be detected by the mottling of the leaves and by line and ringspot patterns.

Celery.—The symptoms of the disease on celery develop on the older and intermediate leaves, but not on the younger ones. Experimentally infected celery does not appear dwarfed or stunted; it seems to be only mildly affected. The symptoms vary considerably.

Some ringspots are formed by pale-green lines or bands, which later become yellow rings or bands. Imperfect rings in the form of semicircles may occur along the margin of the leaflets (plate 2, *A*) ; or they may be circular, oval, or irregularly shaped (plate 2, *B*). The ringspots may be few, or they may occur abundantly on all the leaflets (plate 2, *C*).

There are at least four types of ringspot patterns, according to the arrangement of parts. These may be described as follows: yellow ring or band encircling green tissue (plate 2, *A, B*) ; green ring enclosing a chlorotic center (plate 2, *C*) concentric alternating yellow and green lines surrounding a green area (plate 2, *D;* plate 3, *A*), which is sometimes surrounded by a pale yellow halo (plate 2, *E*).

The chlorotic line patterns also vary. The lines may be broken, composed of dots and dashes, which sometimes surround green tissue (plate 2, *F*) ; or concentric, broken lines may alternate with parallel green lines of tissue enclosing green areas (plate 3, *A*) ; or irregular, yellow bands may encircle green areas (plate 3, *B*) ; or chlorotic tissue may run zigzag, resembling an oak-leaf pattern (plate 3, *C*). Line patterns and ringspots may appear on the same leaflets (plate 2, *D;* plate 3, *A*).

Sometimes on the leaves of celery plants in an advanced stage of the disease, small chlorotic areas are fused, forming large, irregular, yellow areas which surround green spots (plate 3, *D*). Chlorosis may continue (plate 3, *E*), spreading over the leaflets, with the green tissue still remaining (plate 3, *F*). The chlorotic patterns on the outer and intermediate leaves may fade from yellow to white, gradually disappearing as the maturing leaves become entirely chlorotic ; and the plant may then show no further symptoms of disease. Previously noninfective aphid vectors, however, readily recover the virus from such symptomless plants and transfer it to healthy ones.

Large Smooth Prague Celeriac.—Numerous large chlorotic rings encircling green areas appear on the outer leaves of infected plants (plate 4, *A*).

Dill.—Although the virus was recovered from a single plant, no ringspots were observed under the binocular microscope on the finely dissected leaves.

Salad Chervil.—The first symptom on the outer leaves was circular or elliptical, chlorotic areas, which coalesced and formed bands. Some plants showed necrotic spots, commonly along the serrated margin of the leaflets, often followed by purpling, browning, and drying of the outer leaves.

Coriander.—Pale-yellow areas, circular or elliptical, developed along the margin of the leaflets, then became deep yellow, and frequently coalesced to form bands. The leaflet tips were often yellow.

Carrots.—On the oldest leaves of carrot varieties examined under the binocular microscope, the symptoms varied somewhat. Most of the varieties showed small, sunken, chlorotic areas. Sometimes a chlorotic ring occurred, with or without a green center surrounded by cleared veinlets; or solid, irregular, yellow areas were present in the depressions.

Hollow Crown Parsnip.—The outer leaves of infected plants showed circular, pale-green areas, usually with no outer chlorotic rings; the rings when present were not sharply defined.

Long Smooth Parsnip.—The oldest leaves of diseased plants developed circular, pale-green areas with no outer chlorotic rings.

Single or Plain Parsley.—The oldest leaves developed a striking pattern—ringspots, broken yellow lines, zigzag lines resembling an oak-leaf pattern, and green or chlorotic veinbanding (plate 4, *D, E*). Each ringspot was composed of an outer chlorotic ring and an inner green ring, enclosing a chlorotic center.

Curled Varieties of Parsley.—Scattered over the oldest leaves, as observed under the binocular microscope, were extremely small chlorotic circular or irregular areas that coalesced, forming irregular blotches.

Hamburg, or Turnip-Rooted, Parsley.—In the early stages of the disease, the outer leaves developed chlorotic rings enclosing green areas (plate 4, *B*); later they showed broken, chlorotic lines composed of dots, dashes, or streaks. Occasionally clusters of dashes or dots surrounded green areas, forming ringspots (plate 4, *C*). Sometimes chlorosis gradually spreads over the leaflets, leaving only the veins banded with green tissue (plate 4, *C*).

TABLE 1

TRANSMISSION OF VIRUS FROM EXPERIMENTALLY INFECTED POISON HEMLOCK TO HEALTHY CELERY BY HONEYSUCKLE APHID AND BY MECHANICAL INOCULATION TO POISON HEMLOCK AND CELERY

Infected poison hemlock no.	Aphid transmission: celery plants infected, of 5 inoculated	Mechanical transmission	
		Poison hemlock infected, of 5 inoculated	Celery plants infected, of 5 inoculated
1	5	0	0
2	5	0	0
3	4	0	0
4	5	0	0
5	5	0	0
6	5	0	0
Totals	29	0	0
Percentage	*96.7*	*0.0*	*0.0*

SYSTEMIC NATURE OF THE VIRUS

Because the symptoms of ringspot on celery appear only on the outer and intermediate leaves, an experiment was undertaken to determine whether the virus was systemic. All leaves showing symptoms were removed from 10 celery plants, and then previously noninfective honeysuckle aphids were fed on these plants for 1 to 7 days. Lots of 25 aphids were then transferred from each infected plant to each of 5 healthy celery plants. The aphids recovered the virus from the symptomless leaves of the 10 plants and transmitted it to 33 of 50 celery plants, a fact indicating that the virus is systemic. After removal of the aphids, 9 of the 10 plants subsequently developed symptoms again on the outer and intermediate leaves.

INOCULATION EXPERIMENTS

Extract from Diseased Poison Hemlock.—An attempt was made to transmit the virus by mechanical inoculation of extracted sap from experimentally

infected poison hemlock to healthy poison hemlock and celery. Transmission by aphids served to prove the presence of the virus. Table 1 gives the results. Mechanical inoculation of 30 poison-hemlock and 30 celery plants with the juice from 6 experimentally infected specimens of poison hemlock had no effect; yet aphids acquired the virus from each one of the 6 sources of inoculum

TABLE 2

TRANSMISSION OF VIRUS FROM EXPERIMENTALLY INFECTED SINGLE, OR PLAIN, PARSLEY TO PARSLEY BY MECHANICAL INOCULATION AND BY THE HONEYSUCKLE APHID TO CELERY PLANTS

Single or plain parsley no.	Mechanical inoculation: parsley plants infected, of 5 inoculated	Aphid transmission: celery plants infected, of 5 inoculated
1	3	3
2	2	3
3	3	2
4	2	1
5	3	2
6	1	1
7	4	0
8	4	0
9	5	0
10	3	0
11	2	0
12	2	0
13	1	0
14	0	4
15	0	4
16	0	4
17	0	2
18	0	4
19	0	3
20	0	4
21	0	2
22	0	2
23	0	1
24	0	1
25	0	3
26	0	1
27	0	1
28	0	1
Total	35	49
Percentage	*25.0*	*35.0*

and transmitted it to 29 of 30 celery plants, or 96.7 per cent. Thus, although the virus from poison hemlock was recovered readily by aphids, it could not then be transmitted to poison hemlock and celery by the carborundum method of mechanical inoculation.

Extract from Diseased Celery.—The ringspot virus could not be transmitted by mechanical inoculation of sap from infected to healthy celery. As tables 3 and 5 show, 500 healthy celery plants were inoculated mechanically with juice from 100 infected plants without the production of a single diseased

plant. Aphid transmission proved that the virus was present in 72 per cent of the celery plants used as a source.

Extract from Diseased Parsley.—Although poison hemlock and celery plants could not be infected with the ringspot-virus extract by mechanical inoculations, nevertheless, the extract from experimentally infected Single, or Plain, parsley inoculated in healthy parsley produced infection in a low percentage of trials. According to table 2, previously noninfective honeysuckle aphids recovered the virus from 21 of 28 infected parsley plants and infected

TABLE 3

TRANSMISSION OF VIRUS BY ELEVEN SPECIES OF APHIDS AND ATTEMPT TO TRANSMIT THE VIRUS FROM EXPERIMENTALLY INFECTED TO HEALTHY CELERY PLANTS BY MECHANICAL INOCULATION

Aphid	Aphid transmission, celery plants			Mechanical inoculation, celery plants		
	Inoculated	Infected	Per cent infected	Inoculated	Infected	Per cent infected
Celery leaf aphid, *Aphis apigraveolens* Essig	25	14	56.0	25	0	0.0
Celery aphid, *Aphis apii* Theo	25	9	36.0	25	0	0.0
Rusty-banded aphid, *Aphis ferruginea-striata* Essig	25	6	24.0	25	0	0.0
Cotton or melon aphid, *Aphis gossypii* Glover	25	1	4.0	25	0	0.0
Erigeron root aphid, *Aphis middletonii* Thos.	25	4	16.0	25	0	0.0
Bean or dock aphid, *Aphis rumicis* Linn.	25	1	4.0	25	0	0.0
Yellow willow aphid, *Cavariella capreae* (Fab.)	25	7	28.0	25	0	0.0
Lily aphid, *Myzus circumflexus* (Buck.)	25	14	56.0	25	0	0.0
Foxglove aphid, *Myzus convolvuli* (Kalt.)	25	3	12.0	25	0	0.0
Green peach aphid, *Myzus persicae* (Sulzer)	25	5	20.0	25	0	0.0
Honeysuckle aphid, *Rhopalosiphum conii* (Dvd.)	25	18	72.0	25	0	0.0
Total	275	82	....	275	0	....

49 of 140 celery plants, or 35 per cent. The virus was recovered from 13 of 28 infected parsley plants and mechanically transmitted by sap inoculation to 35 of 140 parsley plants, or 25 per cent. Virus was successfully recovered by both aphid and mechanical transmission from only 6 of 28 infected parsley plants. Attempts to use parsley as a source of virus and as a test plant to determine the physical properties of the virus were not encouraging; the experiments were discontinued because of erratic results.

APHID TRANSMISSION OF RINGSPOT VIRUS

Vectors Breeding on Celery.—According to previous observations (Severin and Freitag, 1938) at least 11 species of aphids breed on celery under natural conditions in California, and all these are vectors of the western-celery-mosaic virus. The 11 species (listed in table 3) were tested for transmission of ringspot virus by transferring previously noninfective aphids of each species to 5 infected celery plants. The aphids fed on this material for about 7 days before being transferred to 5 healthy celery plants in lots of 25 per plant. Table 3 presents the results obtained with aphid transmission and also with attempted mechanical transmission.

All 11 aphid species proved capable of transmitting the virus from celery

to celery, but their efficiency varied greatly (table 3). The honeysuckle aphid was found to be the most efficient vector. The melon or cotton aphid, *Aphis gossypii* Glover, and the bean or dock aphid, *Aphis rumicis* Linn., were inefficient, transferring the virus to only 1 plant of 25, or 4 per cent.

As table 3 indicates, 275 celery plants were mechanically inoculated with sap from infected plants used as a virus source in the aphid experiments; but, again, none of them developed symptoms.

Single Aphids as Vectors.—Single specimens of winged and mature wingless aphids of 2 species that breed naturally on poison hemlock, the honeysuckle aphid, *Rhopalosiphum conii* (Dvd.), and the rusty-banded aphid, *Aphis ferruginea-striata* Essig, were tested for their ability to transmit the virus. The winged honeysuckle aphids infected 28 out of 100 celery plants; the mature wingless, only 16 out of 100. With the rusty-banded aphids, the situation was reversed: the winged specimens infected only 2 out of 100 celery plants; the mature wingless, 19 out of 100.

Vectors That Do Not Breed on Poison Hemlock and Celery.—An attempt was made to transmit the ringspot virus by means of 5 species of aphids that do not breed on poison hemlock and celery plants under natural conditions in California. To celery plants infected with ringspot virus, the black peach aphid, *Aphis persicae-niger* Smith, was transferred on peach leaves; the mealy plum aphid, *Hyalopterus arundinis* (Fab.), on prune; and the black cherry aphid, *Myzus cerasi* (Fab.), on cherry. As the leaves wilted and became dried, the aphids moved from them to the foliage of infected celery. Next day the aphids on the infected plants were transferred to 5 healthy celery plants in lots of 25 per plant. Previously noninfective specimens of the cabbage aphids, *Brevicoryne brassicae* (Linn.), and the turnip or false cabbage aphid, *Rhopalosiphum pseudobrassicae* (Davis), reared in the greenhouse, were also used in attempts at transmission. The 5 species tested survived on celery for only a few days, and 4 of the 5 failed to transmit the virus. The false cabbage or turnip aphid infected only 1 of 25 plants inoculated. The 4 aphid species that failed as vectors might possibly produce infections if additional tests were undertaken.

Transmission by Honeysuckle Aphids during Short Feeding Periods.—Efforts to transmit the ringspot virus by feeding each of 112 single honeysuckle aphids 5 minutes on diseased and then 5 minutes on healthy celery were all failures.

Since single honeysuckle aphids did not transmit the virus during 5-minute feeding periods on each diseased and healthy plant, it was decided to test lots of 25 feeding for short periods. The noninfective honeysuckle aphids were fasted for a half hour. Lots of 25 were then fed for 5, 10, and 15 minutes on the infected celery and immediately transferred to healthy plants, where they were fed for the same intervals. At each of the three short feeding periods, 100 lots of 25 aphids were tested. These lots infected only a low percentage of the celery—2 of the 100 celery plants during the 5-minute, 3 of the 100 plants during the 10-minute, and 2 of the 100 plants during the 15-minute feeding periods.

The small number of transmissions obtained by feeding lots of 25 honeysuckle aphids on the diseased celery for short periods made it desirable to

lengthen the period to 1 hour; the aphids were then transferred hourly to 9 successive healthy plants. As table 4 shows, 10 of 12 lots transmitted the virus during the first hour. Two lots failed to infect the first healthy plant; one of these lots infected the second plant, and the other the third. Three lots infected two successive plants on which they fed during the first and second hours. Two lots transmitted the virus once, then failed to transmit it to several successive healthy plants, but finally produced infection during the eighth hour.

Judging from the results, the honeysuckle aphid can infect healthy plants immediately after feeding for short periods on diseased ones. There is no

TABLE 4

TRANSMISSION OF VIRUS BY TWENTY-FIVE PREVIOUSLY NONINFECTIVE HONEYSUCKLE APHIDS FED ONE HOUR ON INFECTED CELERY AND THEN TRANSFERRED HOURLY TO NINE SUCCESSIVE HEALTHY CELERY PLANTS

Lot no.	Infections produced*									Number of celery plants infected
	2d hour	3d hour	4th hour	5th hour	6th hour	7th hour	8th hour	9th hour	10th hour	
1	+	−	−	−	−	−	−	−	−	1
2	+	−	−	−	−	−	−	−	−	1
3	+	+	−	−	−	−	−	−	−	2
4	+	−	−	−	−	−	−	−	−	1
5	+	+	−	−	−	−	−	−	−	2
6	+	−	−	−	−	−	+	−	−	2
7	+	−	−	−	−	−	−	−	−	1
8	+	−	−	−	−	−	−	−	−	1
9	+	+	−	−	−	−	−	−	−	2
10	+	−	−	−	−	−	−	−	−	1
11	−	+	−	−	−	−	+	−	−	2
12	−	−	+	−	−	−	−	−	−	1
Total +	10	4	1	0	0	0	2	0	0	
Percentage	*83.3*	*33.3*	*8.3*	*0.0*	*0.0*	*0.0*	*16.7*	*0.0*	*0.0*	

* The plus sign (+) indicates the production of the disease, and the minus sign (−) shows that no infection resulted.

indication that a certain length of time must elapse between the acquiring of the virus and the infection of a plant. In the last experiment, the honeysuckle aphid usually infected only one or both of the first two healthy plants on which it fed, then lost the capacity to produce infection; it never retained the virus for more than 8 hours. If the tests had been more extensive and had continued somewhat longer, there might have been infections after 8 hours.

Retention of Virus by Aphids.—Two experiments were conducted to determine how long the honeysuckle aphid retained the ringspot virus. The first experiment tested 9 species of aphids that breed on celery under natural conditions. The species tested are listed in table 5. In each test 25 infective aphids reared on diseased celery in the greenhouse were transferred daily to 3 successive healthy celery plants. The aphids were confined on the third celery plant for a week, at the end of which they were killed by fumigation with Nico-fume tobacco paper. With each species, 25 tests were made.

As table 5 shows, the aphids generally lost the infective power during the first 24 hours of the experiment. Of the 225 celery plants on which they fed the

TABLE 5

RETENTION OF VIRUS BY NINE SPECIES OF APHIDS TRANSFERRED DAILY TO THREE SUCCESSIVE HEALTHY CELERY PLANTS, AND ATTEMPT TO RECOVER VIRUS BY MECHANICAL INOCULATION FROM INFECTED CELERY ON WHICH APHIDS WERE REARED

Aphid	Aphid transmission									Mechanical inoculation		
	1st day			2d day			3d to 9th day					
	Plants inoculated	Plants infected	Per cent infected	Plants inoculated	Plants infected	Per cent infected	Plants inoculated	Plants infected	Per cent infected	Plants inoculated	Plants infected	Per cent infected
Celery leaf aphid, *Aphis apigraveolens* Essig	25	15	60.0	25	0	0.0	25	0	0.0	25	0	0.0
Celery aphid, *Aphis apii* Theo	25	19	76.0	25	4	16.0	25	0	0.0	25	0	0.0
Rusty-banded aphid, *Aphis ferruginea-striata* Essig	25	12	48.0	25	1	4.0	25	0	0.0	25	0	0.0
Erigeron root aphid, *Aphis middletonii* Thomas	25	18	72.0	25	1	4.0	25	0	0.0	25	0	0.0
Yellow willow aphid, *Cavariella capreae* (Fab.)	25	1	4.0	25	0	0.0	25	0	0.0	25	0	0.0
Lily aphid, *Myzus circumflexus* (Buck.)	25	18	72.0	25	1	4.0	25	0	0.0	25	0	0.0
Foxglove aphid, *Myzus convolvuli* (Kalt.)	25	14	56.0	25	0	0.0	25	0	0.0	25	0	0.0
Honeysuckle aphid, *Rhopalosiphum conii* (Dvd.)	25	17	68.0	25	1	4.0	25	0	0.0	25	0	0.0
Cotton or melon aphid, *Aphis gossypii* Glover	25	1	4.0	25	0	0.0	25	0	0.0	25	0	0.0
Total	225	115	....	225	8	....	225	0	....	225	0	....

first day, 115, or 51.1 per cent, became infected. The aphids succeeded in infecting only 8, or 3.5 per cent, of the 225 plants the second day; and none of the 9 species infected any celery the following week. The celery aphid was the most efficient vector, whereas the cotton or melon aphid and the yellow willow aphid were the least efficient, infecting only 1 plant out of 25 tested the first day.

According to table 5, no infection resulted when juice from the infected celery used as a source of virus for the aphids was mechanically inoculated

TABLE 6

RETENTION OF VIRUS BY LOTS OF TWENTY-FIVE INFECTIVE HONEYSUCKLE APHIDS REARED ON DISEASED CELERY AND TRANSFERRED HOURLY TO TEN SUCCESSIVE HEALTHY CELERY PLANTS

Lot no.	Infections produced*										Number of plants infected
	1st hour	2d hour	3d hour	4th hour	5th hour	6th hour	7th hour	8th hour	9th hour	10th hour	
1	+	−	+	+	−	−	−	−	−	−	3
2	+	+	+	+	+	−	−	−	−	−	5
3	+	−	+	−	−	−	−	−	−	−	2
4	+	−	−	−	−	−	−	−	−	−	1
5	+	+	+	−	−	−	−	−	−	−	3
6	−	−	+	+	+	−	−	−	−	−	3
7	−	+	+	−	−	−	−	−	−	−	2
8	+	+	−	−	+	−	−	−	−	−	3
9	−	+	+	+	−	−	−	−	−	−	3
10	+	+	+	−	−	−	−	−	−	−	3
11	+	−	−	−	−	−	−	+	−	−	2
12	+	+	−	+	+	−	−	−	−	−	4
13	+	+	−	−	−	−	−	−	−	−	2
14	+	−	+	−	−	−	−	−	−	−	2
15	+	+	−	−	−	−	−	−	−	−	2
16	−	+	−	−	−	−	−	−	−	−	1
17	+	−	−	−	−	−	−	−	−	−	1
18	−	+	−	−	−	−	−	−	−	−	1
Total +	13	11	9	5	4	0	0	1	0	0	..
Percentage	*72.2*	*61.1*	*50.0*	*27.8*	*22.2*	*0.0*	*0.0*	*5.5*	*0.0*	*0.0*	..

* The plus sign (+) indicates the production of the disease, and the minus sign (−) shows that no infection resulted.

into 225 healthy celery plants. The failure of mechanical inoculation has already been discussed under "Inoculation Experiments" (p. 394).

In the second experiment 18 lots of 25 honeysuckle aphids reared on infected celery were transferred hourly to 10 successive healthy plants. As table 6 shows, the aphids infected a higher percentage of celery the first hour than during any later period. The results demonstrate a gradual loss of the virus by the aphids: one lot in this experiment infected a celery plant the eighth hour; none infected any during the ninth and tenth hours.

Loss and Recovery of Infectivity by Honeysuckle Aphids on Inoculated Celery.—An experiment was conducted to determine whether the honeysuckle aphid could recover the ringspot virus from celery before symptoms developed. Five celery plants were inoculated by lots of 100 aphids reared on in-

fected celery. Single aphids were transferred daily for 21 days from 1 inoculated plant to each of 10 healthy ones, and lots of 25 aphids from each of the remaining 4 inoculated plants to each of 4 healthy ones. This experiment was repeated three times, with the results indicated in table 7. The same aphids that were used to infect the plant were used to recover the virus. Previous experiments (p. 399) indicated that the aphids failed to retain the virus for more than two days and, therefore, that any infectivity demonstrated after the second day would be evidence of recovery of virus from the plant rather than retention by the aphids.

TABLE 7

LOSS AND RECOVERY OF INFECTIVITY BY HONEYSUCKLE APHIDS ON CELERY INOCULATED WITH RINGSPOT VIRUS

Number of days after plants were inoculated	Single aphids: plants infected of 10 inoculated			Lots of 25 aphids: plants infected of 4 inoculated		
	Expt. 1	Expt. 2	Expt. 3	Expt. 1	Expt. 2	Expt. 3
2	0	0	0	0	0	0
3	1	1	0	2	1	0
4	1	0	0	2	0	1
5	0	1	1	3	1	2
6	4	2	3	4	2	3
7	4	4	0	4	3	3
8	2	2	0	4	2	2
9	1	1	0	4	1	4
10	3	1	1	4	3	3
11	4	2	3	3	3	3
12	3	1	2	3	2	2
13	6	5	2	4	3	4
14	1	4	5	4	3	4
15	3	5	2	4	4	3
16	5	3	2	3	4	4
17	4	4	5	4	4	4
18	2	5	5	4	3	3
19	4	3	2	3	2	2
20	3	5	3	3	2	3
21	3	5	5	3	3	4
Total	54	54	41	65	46	54
Percentage	*27.0*	*27.0*	*20.5*	*81.3*	*57.5*	*67.5*

As table 7 shows, single aphids and lots of 25 recovered the virus from the original inoculated celery the third day and transferred it to healthy celery. They acquired the virus more readily the sixth and seventh day after inoculation. The symptoms in the original inoculated plants required 21 days to develop. In other experiments the incubation period of the disease varied from 7 to 33 days, with an average of 16.4 days. The results show that the aphids acquired the virus from inoculated plants before symptoms appeared.

Availability of the Virus to Honeysuckle Aphids after Infection.—Experiments were conducted to test whether the virus is more available to the aphids in recently infected celery, or in plants that had been infected for a long time. The methods followed resembled those described under the preceding subheading. Tests were made with single aphids and with lots of 25, and for the

TABLE 8

ABILITY OF SINGLE AND LOTS OF TWENTY-FIVE HONEYSUCKLE APHIDS TO ACQUIRE AND TRANSMIT VIRUS FROM CELERY PLANTS AT INTERVALS FOLLOWING INFECTION

Number of days after plants were inoculated	Single aphids: plants infected of 10 inoculated	Lots of 25 aphids: plants infected of 4 inoculated	Number of days after plants were inoculated	Single aphids: plants infected of 10 inoculated	Lots of 25 aphids: plants infected of 4 inoculated	Number of days after plants were inoculated	Single aphids: plants infected of 10 inoculated	Lots of 25 aphids: plants infected of 4 inoculated	Number of days after plants were inoculated	Single aphids: plants infected of 10 inoculated	Lots of 25 aphids: plants infected of 4 inoculated
2	0	0	61	0	2	121	1	2	181	0	3
3	0	0	62	0	1	122	4	4	182	1	3
4	0	1	63	3	4	123	3	3	183	1	2
5	1	2	64	0	1	124	6	3	184	0	3
6	3	3	65	1	1	125	0	2	185	2	3
7	0	3	66	1	4	126	2	4	186	0	3
8	0	2	67	1	2	127	0	4	187	0	1
9	0	4	68	1	3	128	0	4	188	5	2
10	1	3	69	4	2	129	1	4	189	1	3
11	3	3	70	0	2	130	2	3	190	3	3
12	2	2	71	1	4	131	1	3	191	1	2*
13	2	4	72	1	3	132	0	4	192	0	0*
14	5	4	73	1	3	133	2	3	193	2	2*
15	2	3	74	3	1	134	3	3	194	0	1*
16	2	4	75	3	3	135	3	0	195	3	1*
17	5	4	76	1	1	136	2	4	196	5	2*
18	5	3	77	2	3	137	3	3	197	2	2*
19	2	2	78	3	3	138	3	2	198	1	2*
20	3	3	79	4	3	139	2	4	199	2	0*
21	5	4	80	0	0	140	2	4	200	1	2*
Total	41	54	Total	30	46	Total	40	63	Total	30	40
Percentage	*20.5*	*67.5*	*Percentage*	*15.0*	*57.5*	*Percentage*	*20.0*	*78.8*	*Percentage*	*15.0*	*66.7*

* After the 190th day, only 2 plants were inoculated each day, so that the total number of plants inoculated for the period from 181 days to 200 days was 60 instead of 80 as in other periods.

following four periods after infection: 2 to 21 days, 61 to 80 days, 121 to 140 days, and 181 to 200 days.

Judging from the percentage of transmission shown in table 8 the virus was equally available during the four intervals following infection. Single aphids recovered it regularly during each of the four periods and infected 15.0 to 20.5 per cent of the celery. Lots of 25 infected 57.5 to 78.7 per cent of the celery during the four periods without showing significant differences in ability to acquire the virus.

The aphids while feeding and multiplying on the infected celery under test would repeatedly reinfect the plants. Since, however, the virus is not known to undergo any changes or multiplication within the body of the insect, this fact should not affect the results. The aphids would merely return virus to the leaf from which they had obtained it. To prevent reinfection, the periods during which they were allowed to feed on infected celery would have to be very short; but the low percentage of transmissions obtained during short feeding periods makes such experiments impractical.

SUMMARY

Poison hemlock, *Conium maculatum* L., is the only plant demonstrated to be naturally infected with ringspot.

The host range of poison-hemlock-ringspot virus is limited to the family Umbelliferae. The following plants have been experimentally infected by means of the honeysuckle aphid; celery, *Apium graveolens* L. var. *dulce* DC.; Large Smooth Prague celeriac, *Apium graveolens* L. var. *rapaceum* DC.; dill, *Anethum graveolens* L.; salad chervil, *Anthricus Cerefolium* Hoffm.; coriander, *Coriandrum sativum* L.; varieties of carrots, *Daucus Carota* L. var. *sativa* DC.; Hollow Crown and Long Smooth parsnip, *Pastinaca sativa* L.; and horticultural and botanical varieties of parsley, *Petroselinum crispum* Nym. and *P. crispum* Nym. var. *latifolium*.

Symptoms of the disease consisted mainly of chlorotic line and ring patterns.

The ringspot virus was demonstrated to be systemic in the celery plant. The virus was mechanically transmitted from parsley to parsley in 14.1 per cent of the tests, but could not be thus transmitted from poison hemlock and celery.

Eleven species of aphids that breed on celery under natural conditions were demonstrated to be vectors of ringspot virus.

Four of the 5 aphid species tested that do not breed on celery or poison hemlock failed to transmit ringspot virus.

The honeysuckle aphid transmitted the virus in a low percentage of trials during short feeding periods of 5, 10, and 15 minutes on each diseased and healthy plant.

Previously noninfective honeysuckle aphids, fed for 1 hour on a diseased celery plant and then fed hourly on 9 successive healthy celery plants, usually infected the first healthy plant on which they fed, but only a low percentage of the later plants. Two plants were infected during the eighth hour, but none during the ninth and tenth hours.

Experiments conducted to determine how long the aphids retained the virus demonstrated that 4 species lost it during the first 24 hours on healthy plants, whereas 5 species infected a low percentage of plants during the second 24-

hour period, but none infected any after 48 hours. Infective honeysuckle aphids reared on diseased celery and then transferred hourly to 10 successive healthy celery plants infected the highest percentage of healthy plants during the first hour and gradually lost the infective power. All aphids failed to transmit the virus during the ninth and tenth hours.

The same honeysuckle aphids that had infected celery were able to recover virus from plants 3 days after inoculation. Since the minimum incubation period of the disease in the plant was 7 days, this experiment demonstrated that the aphids could acquire the virus before symptoms developed.

Experiments were conducted to determine the ability of aphids to acquire ringspot virus from celery during four 20-day periods after infection. The aphids acquired the virus as readily during the fourth period, 181 to 200 days after infection, as during the first 20 days.

LITERATURE CITED

Bald, J. G., and G. Samuel.
1931. Investigations on spotted wilt of tomatoes. Austral. Council Sci. & Indus. Res., Bul. 54:1–24.

Brierley, P.
1933. Studies on mosaic and related diseases of dahlia. Boyce Thompson Inst. Contrib. 5:235–88.

Burnett, G.
1934. Stunt: a virosis of delphinium. Phytopathology 24:467–81.

Burnett, G., and L. K. Jones.
1931. Effect of certain potato and tobacco viruses on tomato plants. Washington Agr. Exp. Sta. Bul. 259:1–37.

Fromme, F. D., S. A. Wingard, and C. N. Priode.
1927. Ringspot of tobacco; an infectious disease of unknown cause. Phytopathology 17:321–28.

Gardner, M. W., C. M. Tompkins, and O. C. Whipple.
1935. Spotted wilt of truck crops and ornamental plants. (Abstract.) Phytopathology 25:17.

Hamilton, M. A.
1932. On three new virus diseases of *Hyoscyamus niger*. Ann. Appl. Biol. 19:550–68.

Henderson, R. G.
1934. Occurrence of tobacco ringspot-like virus in sweet clover. Phytopathology 24:248–56.

Henderson, R. G., and S. A. Wingard.
1931. Further studies on tobacco ringspot in Virginia. Jour. Agr. Res. 43:191–207.

Hoggan, Isme.
1933. Some viruses affecting spinach and certain aspects of insect transmission. Phytopathology 23:446–74.

Johnson, E. M.
1930. Virus diseases of tobacco in Kentucky. Kentucky Agr. Exp. Sta. Bul. 306:287–415.
1933. A ringspot-like virus of red clover. Phytopathology 23:746–47.

Johnson, E. M., and W. D. Valleau.
1935. The ring symptoms of virus diseases of plants. Kentucky Agr. Exp. Sta. Bul. 361:239–63.

Johnson, James.
1925. Transmission of viruses from apparently healthy potatoes. Wisconsin Agr. Exp. Sta. Res. Bul. 63:1–12.
1936. Tobacco streak, a virus disease. Phytopathology 26:285–92.

Nelson, Ray.
1930. Infectious chlorosis of the rose. (Abstract.) Phytopathology 20:130.

Price, W. C.
1936. Specificity of acquired immunity from tobacco ringspot diseases. Phytopathology 26:665–75.

Priode, C. N.
1928. Further studies in the ringspot disease of tobacco. Amer. Jour. Bot. 15:88–93.

Rawlins, T. E., and C. M. Tompkins.
1936. Studies on the effect of corborundum as an abrasive in plant virus inoculations. Phytopathology 26:578–87.

Samuel, G., J. G. Bald, and H. A. Pittman.
1930. Investigations on spotted wilt of tomatoes. Austral. Council Sci. & Indus. Res. Bul. 44:1–64.

Severin, H. H. P., and J. H. Freitag.
1938. Western celery mosaic. Hilgardia 11(9):495–558.

Smith, J. Henderson.
1928. The transmission of potato mosaic to tomato. Ann. Appl. Biol. 15:517–28.

Smith, K. M.
1929. Studies on potato viruses. IV. Further experiments with potato mosaic. Ann. Appl. Biol. 16:1–32.
1931. Studies on potato virus diseases. VII. On a ringspot virus affecting solanaceous plants. Ann. Appl. Biol. 18:1–14.
1932. Studies on plant virus diseases. XI. Experiments with a ringspot virus. Its identification with spotted wilt of tomatoes. Ann. Appl. Biol. 19:305–20.

Tompkins, C. M., M. W. Gardner, and H. Rex Thomas.
1938. Black ring, a virus disease of cabbage and crucifers. Jour. Agr. Res. 57:929–43.

Valleau, W. D.
1932. A virus disease of delphinium and tobacco. Kentucky Agr. Exp. Sta. Bul. 327:81–88.
1932. Seed transmission and sterility studies of two strains of tobacco ringspot. Kentucky Agr. Exp. Sta. Bul. 327:43–80.
1932. A virus disease of plums and peach. Kentucky Agr. Exp. Sta. Bul. 327:89–103.

Valleau, W. D., and E. M. Johnson.
1930. The relation of some tobacco viruses to potato degeneration. Kentucky Agr. Exp. Sta. Bul. 309:475–507.

Wellman, F. L.
1934. Identification of *celery virus*. I. The cause of southern celery mosaic. Phytopathology 24:695–725.

Whetzel, H. H.
1915. Diseases of peony. Massachusetts Hort. Soc. Trans. pt. 1:103–12.

White, R. P.
1930. An infectious chlorosis of rose. (Abstract.) Phytopathology 20:130.

Wingard, S. A.
1928. Hosts and symptoms of ringspot, a virus disease of plants. Jour. Agr. Res. 37:127–53.

Plate. 1—Poison hemlock, *Conium maculatum* L., ringspot: *A*, leaflets from healthy control plant grown from seeds; B, chlorotic areas scattered irregularly on the leaflets; *C*, leaflets showing irregular chlorotic areas of various sizes, and green veinbanding; *D*, leaflets showing irregular line patterns, *E*, zigzag lines on some of the leaflets, resembling oak-leaf pattern; *F*, leaflets showing ringspots, each composed of a chlorotic ring encircling a green area.

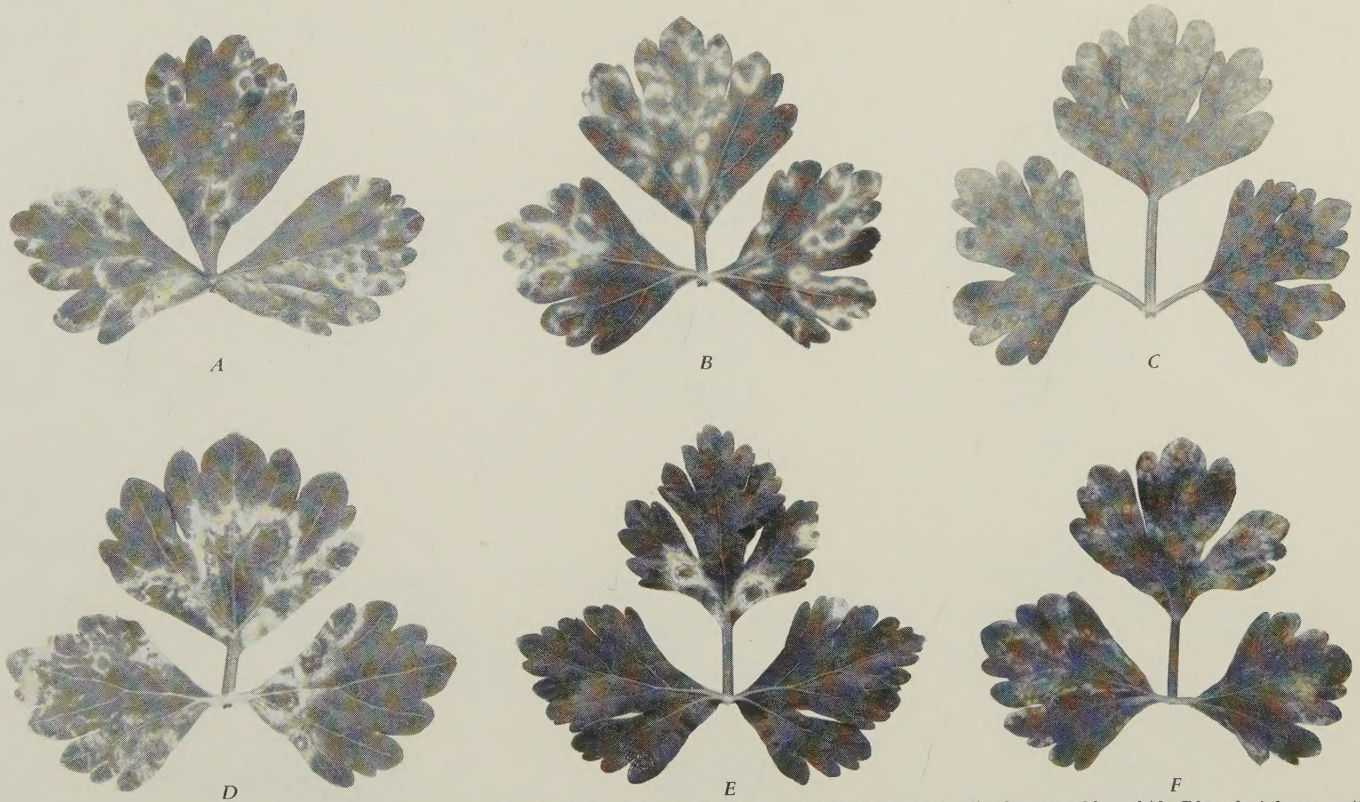

Plate 2.—Symptoms of poison-hemlock ringspot on leaflets of celery plants experimentally infected by the honeysuckle aphid, *Rhopalosiphum conii* (Dvd.): *A*, chlorotic rings encircling green areas and imperfect rings in the form of semicircles along the margin. *B*, Various-shaped ringspots such as circular, oval, or irregular. *C*, Numerous ringspots with green rings enclosing yellow centers. *D*, Several types of ringspots on the leaflets—on the apical leaf, concentric alternating yellow and green lines surrounding green areas; on the leaflet at left, line pattern and ringspot with chlorotic ring encircling green tissue; on the leaflet at right, outer chlorotic ring, inner green ring with a chlorotic center on the basal margin of the leaflet, ringspot with green area embedded in chlorotic tissue near the attachment of the leaflet, and two adjacent ringspots with green rings enclosing chlorotic centers. *E*, Concentric alternating yellow and green lines surrounding a green area within a pale yellow halo. *F*, Broken-line pattern composed of dots and dashes, sometimes enclosing large areas of green tissue.

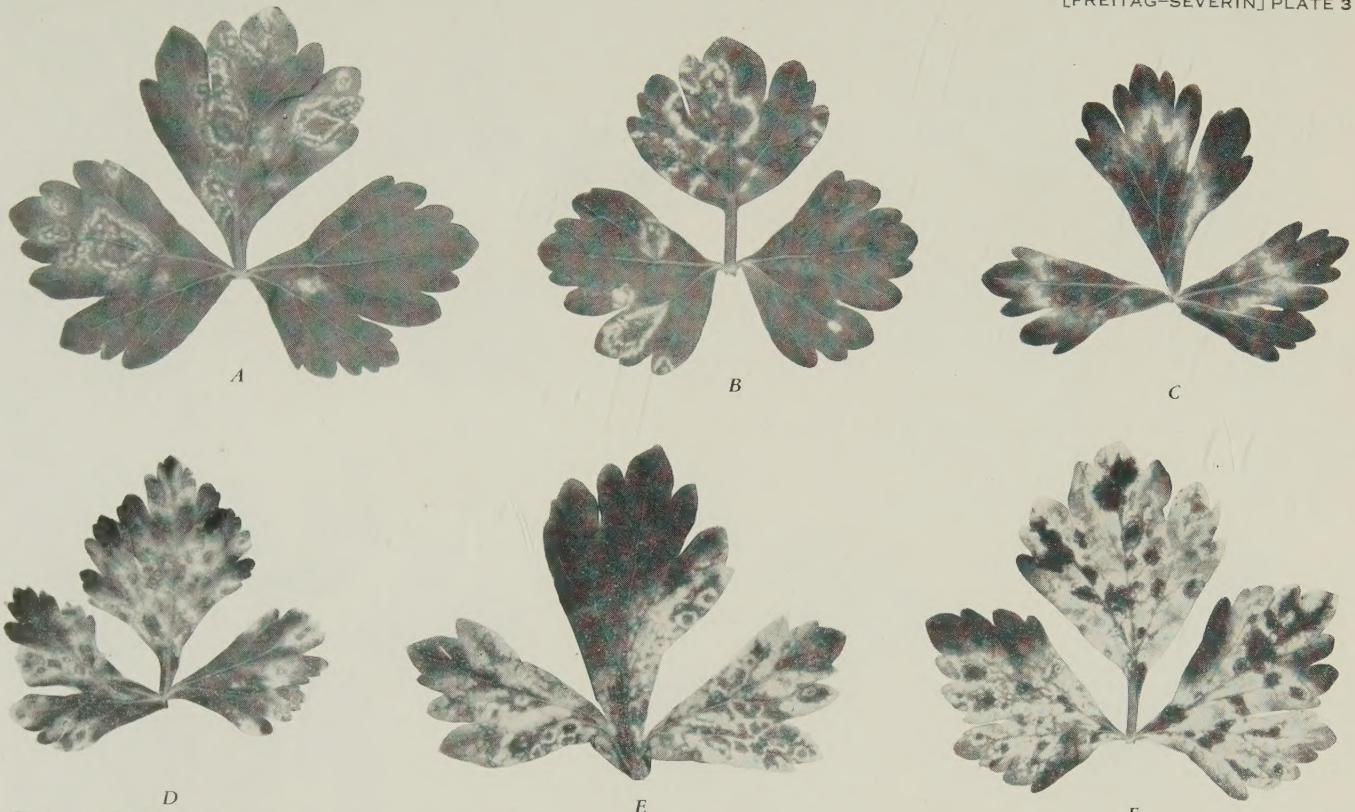

Plate 3.—Symptoms of poison-hemlock ringspot on leaflets of celery plants experimentally infected by the honeysuckle aphid, *Rhopalosiphum conii* (Dvd.): *A*, concentric broken lines alternating yellow with green lines, surrounding large green areas and ringspots with outer chlorotic ring and inner green ring with chlorotic center; *B*, irregular yellow bands enclosing green tissue and chlorotic spots; *C*, zigzag chlorotic tissue resembling oak-leaf pattern; *D*, advanced stage of disease, showing fusion of small chlorotic areas into enlarged irregular yellow tissue in which green spots are embedded; *E*, *F*, advanced stages of disease, showing chlorosis spreading over the leaflets, with green areas remaining.

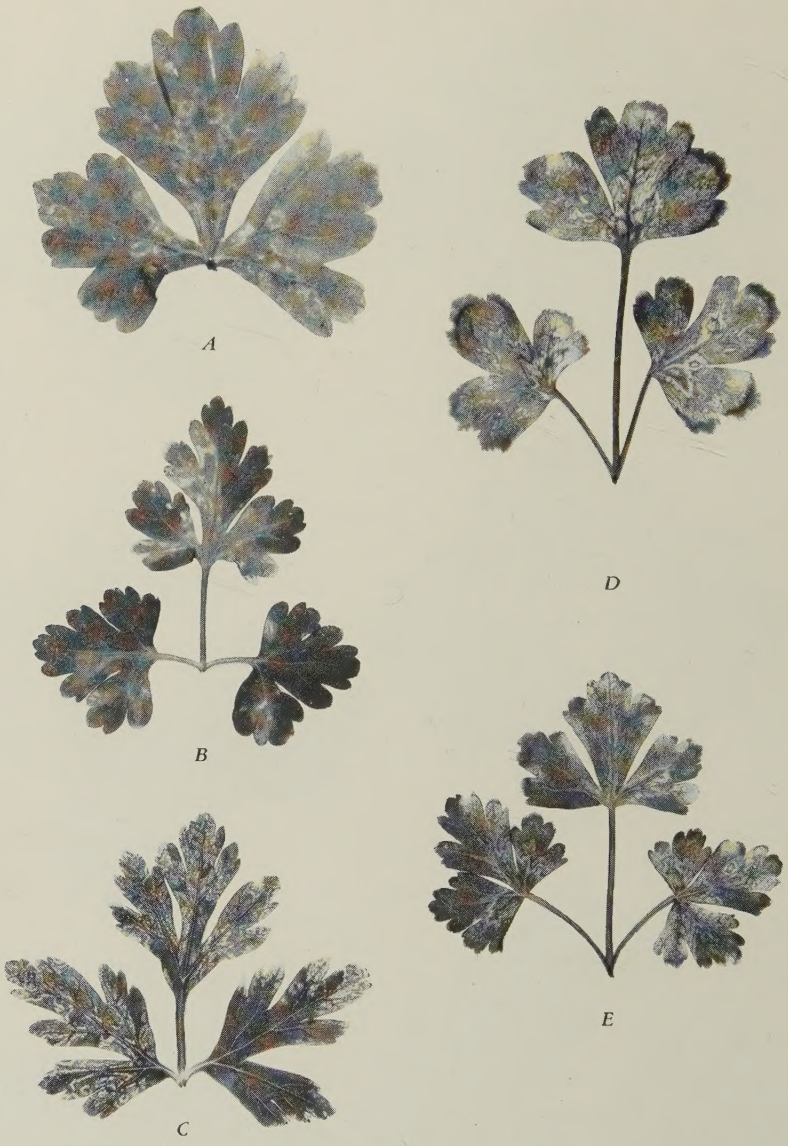

Plate 4.—Symptoms of poison-hemlock ringspot: *A*, leaflets from Large Smooth Prague celeriac, *Apium graveolens* L. var. *rapaceum* DC., showing numerous large chlorotic rings encircling green areas; *B*, early symptoms on leaflets from Hamburg or turnip-rooted parsley, *Petroselinum hortense* Hoffm. var. *radicosum* Bailey, showing chlorotic areas; *C*, later symptoms, showing a few ringspots composed of clusters of chlorotic dots or dashes enclosing green centers and numerous interveinal chlorotic dots, dashes, and streaks often arranged to form broken lines, also green veinbanding; *D*, leaflets from Single or Plain parsley, *Petroselinum crispum* Nym. var. *latifolium*, showing ringspots, each with an outer chlorotic ring, an inner green ring enclosing a chlorotic center, and also chlorotic areas; *E*, leaflets showing a single ringspot, also broken yellow lines, zigzag lines resembling an oak-leaf pattern on the leaflet at the left, and green or chlorotic veinbanding.